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Impact of fertigation type on the anti-dermatophytic potential and its minimal inhibitory drug concentration of petals of *Rosa bourboniana.* Desp

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Abstract
The present study is aimed to explore the potentiality of organic (combination of vermicomposts prepared from cow dung. press mud, tea dust and vegetable wastes in the ratio of (1:1:1:1) and inorganic fertilizer (NPK, 1:2:1 ratio) amendment on the antidermatophytic potentials of petals of Rosa bourboniana for the first time. The plot with no supplements is taken as the control. The experiment was executed in a complete randomized block design (CRBD). An extrapolated value of 6 tons ha $^{-1}$ of amendments were used during the overall 330 days of cultivation period. At the end of the cultivation the petals were extracted with four solvents and were evaluated for anti-dermatophytic screening by disc diffusion method. Dermatophytic strains included in the study were Trichophyton rubrum, Trichophyton mentagrophyte, Candida albicans, Epidermophyton floccosum and Trichophyton tonsurans procured from the Microbial Type Culture Collection and Gene bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Clotrimazole was used as the positive control. All the four extracts showed anti-dermatophytic activity against the selected strains except for the chloroform extract against Trichophyton rubrum and that of ethanolic fractions against Candida albicans. Highest activity was observed with the hexane fractions against Trichophyton tonsurans with 22 mm of zone of inhibition. Highest anti-dermatophytic activity was observed with the samples from the vermicompost treated plots. In the minimal inhibitory drug concentration study the samples from the organic plot was strong enough to inhibit the growth of dermatophytic strains at low concentrations comparing to the other two samples.

Keywords: Rosa bourboniana, vermicompost, inorganic fertilizer, antifungal, disc diffusion method, MIC, clotrimazole

1. Introduction
Rose is an ornamental plant considered as "Queen of flowers" belongs to the family Rosaceae, including 200 species and 18,000 cultivars [1]. The members of this family form a group of erect shrubs and climbing plants, with hispid stems armed with sharp prickles. The flowers are large, attractive, showy and of many colours. The wild species are mostly shrubs, widely distributed in the temperate zones of the northern hemisphere [2]. The history of cultivation of roses dates back to about two thousand years. In the era of the Han dynasty (141- 87 BC) roses were used to decorate the gardens of the royal palace in China [3]. Similarly, roses go far back in history in West Asia and Europe, particularly in eastern countries, where people used to cultivate roses for oil extraction as well as for the purpose of beautification. Further the cultivation practices extended to Egypt, Greece and the Roman Empire from the eastern countries. Rosa moschata and Rosa foetida were perhaps planted for beauty while Rosa damascena was cultivated for the rose oil as well as decoration purpose [4]. Roses are mainly used for showy purposes (both flowers and hips) and for extracting rose oil. From ages, humans have been involved to make new scent by using the foliage and bloom for the enhancement of his surroundings. The utilization of Damask rose dates back to 1500 years ago. The Iranian peoples call the rose as flower of Prophet Muhammad due to its nice fragrance [5]. Roses are widely used in perfumes, creams, soaps, lotions, rose concentrate, rose absolute and other cosmetic products [6]. Petals are used as flavoring ingredients in various food products like cakes, cookies, confectionaries, sweets, ice creams, jams, syrups, for the preparation of gulkand and in making jams.

1.1 Cultivation practices of rose
Rose is the most ancient and popular flower grown the world over. It is a versatile plant adapted to varying climatic conditions.

In India, roses are cultivated in about 6,000 ha area. Karnataka, Tamil Nadu, Maharashtra, Bihar, West Bengal, Uttar Pradesh, Gujarat, Haryana, Punjab, Jammu and Kashmir, Madhya Pradesh and Andhra Pradesh are major rose-growing states in the Nation. Roses require full sunlight or light at least for 6 hours preferably in the forenoon, if not during the whole day. Protection from strong winds is also necessary.

Generally, roses are cultivated in rectangular beds but it may be oval, circular or of irregular shapes depending upon design. It is always better to plant roses in beds than growing individually. Roses are planted in pits with appropriate distances in a bed and while planting the budded plants, the bud union is kept slightly above the ground level. However, planting above this period bushes do not produce good flowers and the flowering is delayed. Roses may be planted from October to December in Tamilnadu and late-May to June in Karnataka and Maharashtra states. September - December or even up to February is ideal planting time in the eastern plains of Bihar and West Bengal. In northern hills, the planting may be taken up in October - November or March - April. As a general rule, planting was not done in either very hot weather or during heavy rains.

1.2 Plants and Dermatophytes

Millions of people throughout the world are affected by superficial fungal infections, which are the most common skin diseases. These infections, which occur in both healthy and immune-compromised persons, are caused mainly by dermatophytes namely Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton verrucosum, Trichophyton tonsurans, Candida albicans, Epidermophyton floccosum and Microsporum nanum. Increasing social and health implications caused by dermatophytes means there is a constant striving to develop safe and new natural antifungal agents to cure human fungal disorders caused by dermatophytes. Many skin diseases such as, tinea and ringworm caused by dermatophytes are existing in tropical and semitropical areas. In general, these fungi live in the dead and top layer of skin cells of moist areas of the body and cause only a minor irritation. Other types of fungal infections could be more serious. They can penetrate into the cells and cause itching, swelling, blistering and scaling [7].

Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses [8]. Dermatophytoses are one of the most frequent skin diseases of human, pets and livestock [9].The disease is widely distributed all over the world with various degrees and more common in men than in women. There are three genera of mould that cause dermatophytosis. These are Epidermophyton, Trichophyton and Microsporum. Contagiousness among animal communities, high cost of treatment, difficulty of control and the public health consequences explain their great importance [10].

The use of systemic drugs is limited to treat man or animal due to their high toxicity and problems of residues in products intended for human consumption [11]. Different treatments have been recommended to control dermatophytes. In general, pharmacological treatment option includes antifungal agents [12], but recently the use of some natural plant products has been emerged to inhibit the causative organisms. They are safe to human and the ecosystem than the synthetic antifungal compounds, and can easily be used by the public who used them for thousands of years to enhance flavor and aroma of foods as well as for its economic value [13].

Plant extracts and their essential oils show antifungal activity against a wide range of fungi [14]. Several studies have shown the efficacy of plant based drugs in the treatment of diseases caused by dermatophytes. The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance against skin diseases. In addition, plant extracts might have inhibitors to enzymes from the invading pathogens, and the effects of different phenolic compounds on the germination and growth of many fungal pathogens have been reported [15].

A number of reports are available *in vitro* and in vivo efficacy of plant extract against plant and human pathogens causing fungal infections [16]. The activity of plant extract against dermatophytosis i.e. the superficial infections of skin or keratinised tissue of man and animals can be very well visualized from many studies [17].They reported the activity of plant extracts against 88 clinical isolates of dermatophytes which includes Microsporum cannis, Microsporum audouinii, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton violaccum, Trichophyton simi, Trichophyton verrucosum, Trichophyton erinacci and Epidermophyton floccosum by agar dilution technique.

With regard to the antifungal activity, petals of Rosa damascena were found to be effective against Penicillium notatum, Aspergillus niger and Candida albicans. There was not much work reported on the efficacy of rose petal extract on dermatophytes. Hence, the petal extracts of Rosa bourboniana is proposed to evaluate its antifungal activity against strains commonly causing skin diseases in human beings. With this view petal extracts of Rosa bourboniana was used to evaluate its activity against fungal strains commonly causing skin diseases in human beings.

2. Materials and Methods

2.1 Cultivation of Rosa bourboniana. Desp

Cultivation of Rosa bourboniana. Desp was done based on the methods described by [18]. Cultivation study was done from January 2012 to February 2014. During the cultivation period plants were fertigated with organic and inorganic amendments.

2.2 Area selected for the study

The study area selected for the present study is located in the Gummidipoondi (National Highway NH5) Panchayat union of Thiruvallur District in Tamilnadu, India.

2.3 Geographical location of the study area

The site selected for study is a small agricultural town located at 13.41° North latitude, 80.12° East longitude and 17 meters elevation above the Mean Sea Level (MSL) located at a distance of about 42 Km from Chennai, Tamilnadu, India.

2.4 Climatic conditions of the study area

The average temperature in the study area ranged between 35 ºC – 40 ºC in summer and 27 ºC – 30 ºC in winter. April, May, June and July are the months with maximum temperatures while November, December and January are the months with minimum temperatures. The study area received rainfall in two seasons namely South West Monsoon prevailing from June to September (451.6 mm) and North East Monsoon (589.3 mm) prevailing from October to December every year.

2.5 Preparation of land for cultivation of Rosa bourboniana

The land selected for cultivation was well ploughed and leveled prior to cultivation. The place selected for cultivation was such that the plants can get proper sunlight for at least 6 hours in a day in every season avoiding shade beneath trees and root competition with those of rose plants for available nutrients and moisture. Double-dig bed raise method was adopted for the cultivation of Rosa bourboniana. The top soil was removed, mixed and tiled to a depth of about 24 hours facilitating aeration to roots, good drain and easy penetration of roots deeper into the soil. This was done to remove rocks and debris that could obstruct root growth. Double-digging provided a reservoir of steady nutrients and sufficient water which can be accessed by rose plants deeper roots. A tractor was employed for leveling the cultivation bed. Both clockwise and anti-clockwise mode of leveling was done during the preparation of cultivation bed. Pebbles and rocks were handpicked manually. Weeds, grasses and other herbs were removed that colonized the field. The cultivation bed was leveled in such a way to ensure proper drain during rainy season.

2.6 Plot preparation: Cultivation bed - Field experimental design and treatment

The experimental plot was a completely randomized block design (CRBD). A plot was designed in the prepared cultivation bed. The size of the study plot was in an area of 10,000 sq.ft with equal length and width. Three rows were partitioned so as to plant rose siblings in single line pattern. Each row was planted with twenty siblings of Rosa bourboniana. Plants were planted at a spacing of 60 cm × 30 cm. The reason for maintaining space is to avoid collision of plants and to ensure proper aeration when it attains a bushy nature. The plot was designed in such a way to prevent nutritional mixing with the adjacent rows. Each row is designated for the application of its respective amendments and easy application of vermicompost and chemical fertilizer to the study plot. T1 is the control plot, T2 is the organic plot (Mixture of vermicomposts prepared from cow dung. press mud, tea dust and vegetable wastes in the ratio of (1:1:1:1) and T3 is the inorganic plot (Chemical fertilizer, NPK in 1:2:1 ratio). An extrapolated value of 6 tons ha $^{-1}$ of amendments were used during the overall 330 days of cultivation period. The experiments were done in triplicates. The cultivation bed of Rosa bourboniana was irrigated daily during peak summers and as per requirements during rainy season. Irrigation was done by canals constructed from bore wells to the study plot.

2.7 Collection of flower material

For anti-dermatophytic analysis fully opened floral blooms were plucked in the early hours of dawn and was collected in separate labeled wet cloth bags and carried to the PG & Research Department of Botany, Pachaiyappa's college, Chennai, India for further studies.

2.8 Processing of floral material for solvent extraction

The plucked flowers were rinsed in distilled water to get rid of contaminants adhering to the petals. The petals were drawn and air-dried under shade for a week at room temperature. The dried petals were segregated and pulverized to fine coarse powder in a blender and sieved through 1 mm sieve.

2.9 Preparation of crude extracts

Floral extracts were prepared by serial extraction involving successive extraction with organic solvents of increasing polarity starting from a non polar to a polar solvent (Hexane, Chloroform, Ethyl acetate and Ethanol). About 100 grams of weighed sieved petal powder was extracted with 1000 ml of each solvent twice with overnight incubation at room temperature for 48 hours. The individual extracts were filtered using Whatmann filter paper No. 1. The filtrate was processed in a vacuum evaporator under reduced pressure to recover the excess solvents for further use. The sticky extract obtained was dried in an oven at 32ºC and stored in vials at 4 ºC for further analysis.

2.10 Selection of test organisms for anti - dermadophytic activity

Fungal test organisms included in the study were five dermatophytic strains which includes Trichophyton rubrum (MTCC NO: 3272), Trichophyton mentagrophytes (MTCC NO: 8476), Candida albicans (MTCC NO: 7315), Epidermophyton floccosum (MTCC NO: 7880) and Trichophyton tonsurans (MTCC NO: 8475). The highly pathogenic microorganisms were procured from the Microbial Type Culture Collection and Gene bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.11 Growth and maintenance of fungal test organisms for study

The procured dermatophytic strains was established by culturing on Potato dextrose agar (PDA - HIMEDIA, Mumbai, India) slants and incubated at 37 ºC in an incubator for 48 hours. The viability is further maintained by frequent culturing in fresh medium once in a week and stored at 4 ºC until used in experiments.

2.12 Testing of Anti-dermatophytic activity by disc diffusion method

Sterile PDA plates were seeded with respective inoculum at 1×10^7 cells/ml and a lawn culture was established in the petri dishes. Sterile discs were loaded with 25µl of each extract dissolved independently in DMSO at a concentration of 1 mg/ml [19]. The loaded discs were impregnated over the established lawn culture aseptically and the plates were incubated at 37 ºC in an incubator for 48 - 72 hours. Zone of inhibition (mm in diameter) were measured and the readings were taken as the antimicrobial activity against the test pathogen for the particular extract. Clotrimazole at concentrations of 30µg/disc were used as the positive control drug. Each assay was repeated thrice and the average values for inhibition zones was recorded and compared with the standard reference antibiotic used [20].

2.13 Minimum inhibitory concentration for the selected dermatophytic strains (MIC)

The minimum inhibitory concentration (MIC) for the fungal strains was determined by using the serial dilution technique using 96 - well microtitre plates [21]. 12 wells in each row of the plate were filled with sterilized 0.1 ml of Potato dextrose broth (HiMedia, Bombay). Column wells 1 - 7 were loaded with a mixture of Nutrient broth (HiMedia, Bombay) and the respective floral extracts diluted serially in a concentration range from 0.25 µl to 5 µl/well. Well 8 served as the growth control. The loaded titre plates were incubated at 37 ºC for 48 - 72 hours and observed for turbidity formation using optical readings at 600 nm using 0.1% alamar blue staining using Beckman DU-70 UV-Vis Spectrophotometer. The results were analyzed in triplicates. The wells in which no turbidity

was observed (no growth of colonies) was taken as the MIC of the particular floral extract for the particular strain. The activity at a concentration of < 50 µg/well of extract was considered as the strongest MIC activity for the particular extract.

3. Results

The comparative study results of the anti-dermatophytic activity of the selected three plots are depicted in Table 1, Fig.1,2 and 3. The hexane extract of the T2 plot produced the highest activity with 16 mm of zone of inhibition followed by the T3 plot with 14 mm and the least zone of inhibition with 10 mm in the T1 plot for the dermatophyte Trichophyton rubrum whereas, no activity was observed with the chloroform extract in all the plots. The ethyl acetate fractions of the flowers from the selected T2 plot and the T3 plot showed a more or less similar activity with 18 mm and 17 mm of zone of inhibitions. While the T1 plot was recorded with only a least of 14 mm of zone of inhibition against Trichophyton rubrum. Surprisingly the ethanolic fractions of the flowers from the T2 plot showed the highest activity of zone of inhibition with 14 mm followed by the fractions of the T1 plot with 11 mm and the least of zone was observed in the T3 plot with 8 mm. The standard drug clotrimazole was found to show an activity of 23 mm of zone of inhibition against Trichophyton rubrum.

For the strain Trichophyton mentagrophytes, highest activity of zone of inhibition was measured as 18 mm for the hexane extract, 15 mm for the chloroform extract and 21 mm for the ethyl acetate extract obtained with the floral extracts of the T2 plot (Table 1). Moderate level activity was observed with the floral extracts of the T3 plot with ranges of 15 mm for the hexane extract, 12 mm for the chloroform extract and 19 mm for the ethyl acetate extract. Whereas the least activity was observed with the floral extracts of the T1 plot with 13 mm for hexane extract, 10 mm for the chloroform extract and 16 mm for the ethyl acetate extract respectively for the strain Trichophyton mentagrophytes (Table 1). Further, the ethanolic fractions from the T2, T3 and the T1 plots recorded inhibition of 7 mm, 3 mm and 5 mm respectively against Trichophyton mentagrophytes.

The standard synthetic antifungal drug showed an activity of 23 mm of zone of inhibition for the same. The hexane fractions of the T2 plot and the T3 plot showed on a slight variation in the zone of inhibition against Candida albicans with 16 mm and 17 mm respectively. The least record was observed with the fractions of the T1 plot corresponding with 12 mm diameter of zone of inhibition (Table 1). The chloroform and the ethyl acetate fractions of the T2 plot showed the highest activity against Candida albicans with 14 mm and 20 mm of zone of inhibition. Second highest activity was observed with the fractions of the T1 plot with 12 mm and 16 mm of inhibition respectively. The ethanolic fractions of all the three plots did not show any activity against Candida albicans wheras a 17 mm of zone of inhibition was observed with the standard synthetic drug against Candida albicans.

Further, the hexane fractions of the T2 plot showed the highest antidermatophytic activity against Epidermophyton floccosum with 20 mm of zone of inhibition followed by the fractions of the T3 and T2 plots with 18 mm and 16 mm respectively (Table 1). The chloroform extract of the T8 plot showed the highest zone of inhibition with 9 mm followed by a more or less moderately equal activity among the fractions of the T1 and T3 plots with 6 mm and 5 mm respectively. The ethyl acetate fractions of the flowers of the T3 plot showed the highest activity of zone of inhibition against Epidermophyton floccosum with 14 mm zone of inhibition. In this case interestingly the fractions from the T2 plot showed only a diameter of 12 mm of zone of inhibition against Epidermophyton floccosum and the T1 plot recorded for the least with only 9 mm of zone of inhibition. While the ethanolic fractions of the flowers from the T2 plot and the T1 plot inihibited a more or less similar activity with 9 mm and 8 mm, the T3 plot was recorded with only a 5 mm of zone of inhibition. For Epidermophyton floccosum the synthetic positive control drug showed an activity of 24 mm diameter of zone of inhibition.

For Trichophyton tonsurans, the hexane extract of the flowers from the T2 plot showed the highest antifungal activity with 22 mm zone of inhibition whereas the T1 plot and the T3 plot recorded only 19 mm and 16 mm of zone of inhibition. While the chloroform extracts of the flowers of the T2, T3 and T1 plots recorded with 18 mm, 15 mm and 14 mm of zone of inhibition respectively (Table 1). Among the ethyl acetate fractions of the three plots, the T2 plot was recorded for the highest antidermatophytic activity of 20 mm which is significantly higher than the T1 plot which provoked 15 mm of zone of inhibition and highly significant than the T3 plot which provoked only a 11 mm of zone of inhibition. Interestingly, for the ethanolic extract, highest antidermatophytic activity was observed with the extracts of the T3 plot with 14 mm of zone of inhibition, the fractions from the T2 plot and the T1 plot recorded 12 mm and 11 mm activity only. The positive control drug showed 19 mm of zone of inhibition against Trichophyton tonsurans in all the plots (Table 1).

The minimum inhibitory drug concentration for dermatophytes showed a wide range of differences within the samples of the control, organic and inorganic plots and the results are stated in Table (2). Among the hexane extract, the samples from the T8 plot required 0.50 µg/ml concentration of drug to inhibit the growth against Trichophyton rubrum, but the samples from the T3 plot and the T1 plot required 1.00 µg/ml concentrations for the complete inhibition of the dermatophytic strain. The chloroform extract of the flowers from all the three plots were found to be resistive against Trichophyton rubrum. A minimum of 0.25 µg/ml of ethyl acetate fractions were enough to inhibit the growth of Trichophyton rubrum, whereas a concentration of 0.50 µg/ml was required for the samples from T3 and T1 plot to inhibit the dermatophtic strain. Among the ethanolic fractions, the samples from the T2 plot inhibited the same strain at 0.25 µg/ml concentration, while that from the T3 plot and T1 plot required 1.00 µg/ml and 0.50 µg/ml concentration of drug to inhibit the fungal growth. The positive control synthetic drug required a minimum concentration of 0.125 µg/ml to inhibit the growth of Trichophyton rubrum.

For Trichophyton mentagrophytes the T2 plot required a minimum of 0.50 µg/ml concentration of the hexane extract for inhibiting, while a minimum of 1.00 µg/ml concentration was required for the samples from the T3 plot and a minimum concentration of 0.75 µg/ml was required to inhibit the growth of the Trichophyton mentagrophytes (Table 2). Among the chloroform extract a minimum of 0.25 µg/ml concentration of extract sample from the T2 plot, a minimum of 0.75 µg/ml of concentration of sample from the T3 plot and a minimum of 1.00 µg/ml concentration of sample extract

from the T1 plot was required to inhibit the growth of Trichophyton mentagrophytes.

Among the ethyl acetate extract, the sample from the T2 plot and the T3 plot inhibited Trichophyton mentagrophytes at a minimum concentration of 0.50 µg/ml whereas the sample from the T1 plot inhibited the growth only at a minimal concentration of 0.75 µg/ml. For the ethanolic fractions, a minimum concentration of 0.25 µg/ml of extract from the T2 plot and a minimum of 0.75 µg/ml concentration of sample from both the T3 and T1 plots was required to inhibit the growth of Trichophyton mentagrophytes and the positive control inhibited the strain at a minimal concentration of 0.125 µg/ml.

For Candida albicans, for the hexane extract a minimum of 0.75 µg/ml concentration of sample from the T2 and T3 plot is required to inhibit the fungal growth, whereas, a minimum concentration of 1.25 µg/ml of extract was required for the samples from the T1 plot. Among the chloroform extract, a minimum of 0.50 µg/ml concentration of T2 sample was required to inhibit the growth of Candida albicans, while a minimum concentration of 1.00 µg/ml of samples from the T3 and T1 plot was required to inhibit the growth of the same strain (Table 2). With the ethyl acetate extract, the sample from the T2 plot required a minimum of 0.25 µg/ml of sample concentration to inhibit the growth of Candida albicans. While the samples from the T3 plot and the T1 plot required a minimum of 1.00 µg/ml and 0.50 µg/ml of concentrations of samples respectively. Interestingly the ethanollic fractions from the selected three plots were found to be resistive against Candida albicans. The positive control drug inhibited the growth of Candida albicans at a uniform concentration of 0.25 µg/ml on all the samples.

For the strain Epidermophyton floccosum, the hexane fractions for the T2 and T1 plot required a minimum concentration of 0.50 µg/ml to inhibit the growth, while the samples from the T3 plot inhibited the growth only at a concentration of 0.25 µg/ml. With the tested samples of chloroform extract, a minimum of 0.25 µg/ml concentration from the samples of the T2 plot was required to inhibit the growth whereas a minimum of 0.50 µg/ml of concentration from the samples of the T3 and T2 plot was required to arrest the growth of Epidermophyton floccosum. Interestingly the ethyl acetate fractions from all the three selected plots required a same minimal inhibitory concentration of 0.75 µg/ml to inhibit the growth of Epidermophyton floccosum (Table 2). In the ethanolic fraction, the samples from the T2 plot and the T1 plot required a minimum of 0.50 µg/ml of concentration to inhibit the growth of strain Epidermophyton floccosum. Whereas, a minimum concentration of 1.00 µg/ml from the samples of the T3 plot was required to inhibit the growth of Epidermophyton floccosum. The synthetic drug inhibited the same strain at a minimum concentration of 0.125 µg/ml.

For the dermatophyte Trichophyton tonsurans, the hexane fractions from the T2 and T3 plot showed inhibitory activity at a minimal concentration of 0.50 µg/ml whereas the samples from the T1 plot required a minimal of 1.00 µg/ml to inhibit Trichophyton tonsurans (Table 2). Likewise in the chloroform fractions, the samples form the T2 and T3 plot showed inhibitory activity at the same minimum concentration of 0.75 µg/ml. and the sample from the T1 plot inhibited the fungal strain at a minimum concentration of 1.00 µg/ml. Among the ethyl acetate fractions, all the three samples inhibited at three different minimum concentrations respectively. In the ethanolic fractions, the samples of all the three selected plots inhibited the growth of Trichophyton tonsurans at the same minimum concentrations of 0.50 µg/ml. The synthetic drug worked at a minimum concentration of 0.25 µg/ml against Trichophyton tonsurans. In general, the flower samples from the T2 plot inhibited the growth of dermatophytes with lesser concentrations when compared to the T3 and the T1 plot.

Table 1. Comparative anti-dermatophytic activity of flowers of Rosa bourboniana from the Control (T1), Organic (T2) and Inorganic (T3) plots

Tested pathogens	Zone of inhibition (mm)												
	Hexane extract			Chloroform extract			Ethyl acetate extract			Ethanol extract			Positive control
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	(Clotrimazole) 30 µg/disc
Trichophyton rubrum	10	16	14	-	-	-	14	18	17	11	14	8	23
Trichophyton mentagrophytes	13	18	15	10	15	12	16	21	19	5	7	3	23
Candida albicans	12	16	17	12	14	11	16	20	13	-	-	-	17
Epidermophyton floccosum	16	20	18	6	9	5	9	12	14	8	9	5	24
Trichophyton tonsurans	19	22	16	14	18	15	15	20	11	11	12	14	19

*Data were recorded during the peak period of flowering. Results represent mean of three replicated experiments,
* Includes 0.5 cm of disc diameter,
*Concentration of plant extract - 1mg dissolved in 1 ml of DMSO,
* Loaded volume - 25 µl

Table 2. Comparative minimum inhibitory concentration (MIC) values of flowers of Rosa bourboniana from the Control (T1), Organic (T2) and Inorganic (T3) plots against selected dermatophytic strains

Tested pathogens	Concentration (µg/ml)												
	Hexane extract (µl)			Chloroform extract (µl)			Ethyl acetate extract (µl)			Ethanol extract (µl)			Positive control (Clotrimazole)
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	(µl)
Trichophyton rubrum	1.00	0.50	1.00	-	-	-	0.50	0.25	0.50	0.50	0.25	1.00	0.125
Trichophyton mentagrophytes	0.75	0.50	1.00	1.00	0.25	0.75	0.75	0.50	0.50	0.75	0.25	0.75	0.125
Candida albicans	1.25	0.75	0.75	1.00	0.50	1.00	0.50	0.25	1.00	-	-	-	0.25
Epidermophyton floccosum	0.50	0.50	0.25	0.50	0.25	0.50	0.75	0.75	0.75	0.50	0.50	1.00	0.125
Trichophyton tonsurans	1.00	0.50	0.50	1.00	0.75	0.75	1.75	1.00	1.25	0.50	0.50	0.50	0.25

*Data were recorded during the peak period of flowering. Results represent mean of three replicated experiments

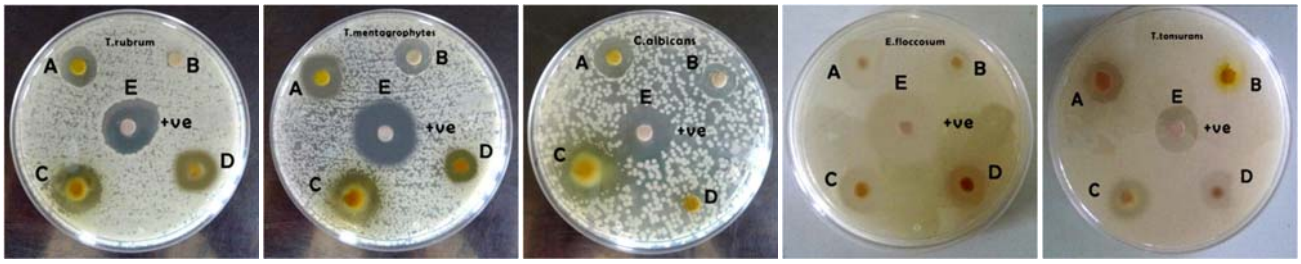


Fig 1: Anti-dermatophytic efficacy of flowers of Rosa bourboniana from the Control plot (T1)

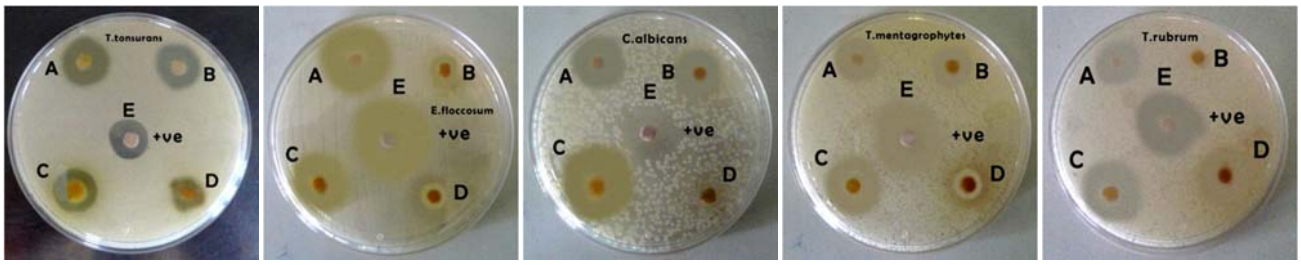
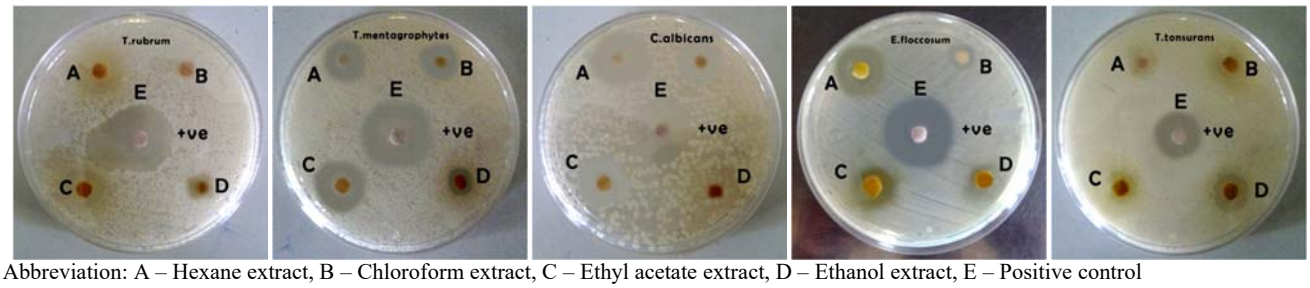


Fig 2: Anti-dermatophytic efficacy of flowers of Rosa bourboniana from the Organic plot (T2)



Abbreviation: A – Hexane extract, B – Chloroform extract, C – Ethyl acetate extract, D – Ethanol extract, E – Positive control

Fig 3: Anti-dermatophytic efficacy of flowers of Rosa bourboniana from the Inorganic plot (T3)

4. Discussion

Dermatophytes are group of closely related fungi comprising of 40 identified species in the dermatophytic genera that include Trichophyton, Microsporum and Epidermophyton documented in literature as potential etiological agents of dermatophytosis [22]. Humid weather, over population and poor hygiene are the ideal conditions for the growth of dermatophytes [23]. Dermatophytes represent the prevailing type of fungi that cause infection of the skin, hair and nails [24]. These infections lead to a variety of clinical manifestations including Tinea capitis, Tinea pedis, Tinea corporis, Tinea cruris and Majocchi's granuloma. These dermatophytes respond differently to different conventional antifungal agents, which have a tendency to reoccur in the same area or other ones [25]. The most commonly used synthetic antifungal agents include Clotrimazole, Miconazole, Flucanozole, Itraconazole and Ketoconazole with many side effects.

The use of plants as a source of medicine to treat infectious disease dates back to history of mankind as a result of which nearly all cultures and civilization from ancient times to the present day have used herbal medicines to cure infections [26]. Antifungal activities of some plants have been reported by various researchers throughout the world [27 – 30]. Due to lack of efficacy, side effects and resistance associated with some of the existing drugs, much of the attention has been paid to plant extracts to treat fungal infections. A review of literature indicates that not much work has been done with the petals of Rosa bourboniana.

The present work with the petals of Rosa bourboniana showed highly significant results against the selected dermatophytes. In general, all the three samples showed a highly significant activity while the samples from T2 plot recorded for the highest activity against all dermatophytes tested in study. The second highest activity was observed with the samples from the T3 plot against Trichophyton rubrum (17mm), Trichophyton mentagrophytes (19 mm), Candida albicans (17 mm) and Epidermophyton floccosum (18 mm). The least range of activity was observed with the samples from the T1 plot against Trichophyton rubrum (14mm), Trichophyton mentagrophytes (16 mm), Candida albicans (16 mm) and Epidermophyton floccosum (16 mm). While against Trichophyton tonsurans second highest activity was observed with the samples from the T1 plot (19 mm) while the least was obtained for the samples from the T3 plot with 16 mm respectively. Interestingly for Candida albicans highest activity was obtained with the ethyl acetate fractions of the samples from the T2 and T1 plots. While the hexane extract was recorded for the highest activity against Candida albicans for the sample from the T3 plot. The solubility and medicinal property changed within the samples with the type of fertilizer amended for cultivation. Only the ethanolic fractions of the three samples did not show any activity against Candida albicans. The standard drug clotrimazole was found to be with more or less similar activity against the selected dermatophytes. However with some solvent fractions the activity was less comparing to the used plant extract. In the increased concentration, the activity may be increased.

In the study, the chloroform and ethyl acetate samples from the T2 plot gave 14 mm and 20 mm of zone of inhibition

against Candida albicans at a concentration of 1 mg/ml, while the same chloroform and ethyl acetate extract of the flowers of Mirabilis jalapa gave an activity of 12 mm and 11 mm of zone of inhibition against Candida albicans even at higher concentrations of 200 mg/ml of concentration [31]. While no activity against Candida albicans was reported in the chloroform extracts of the flowers of Andrographis paniculata [32]. In an another study, even with the high polar methanolic fractions of flowers of Cassia alata did not shown any activity against Candida albicans and Trichophyton mentagrophytes [33].

The present study showed the positive efficacy of petals of Rosa bourboniana against skin infection causing Candida albicans. The ethanolic fractions of the samples from the T2 plot showed an activity range of 14 mm against Trichophyton rubrum, 7 mm against Trichophyton mentagrophytes and 12 mm against Trichophyton tonsurans. In a previous study even with the synergitics of methanol and ethanol extracts of Citrus limon showed an activity of 12 mm against Trichophyton rubrum while it was resistant to Trichophyton mentagrophytes and Trichophyton tonsurans. With Aloe barbadensis, it was resistant to Trichophyton rubrum and showed 11 mm and 13 mm of activity against Trichophyton mentagrophytes and Trichophyton tonsurans. Hibiscus rosasinensis was resistant to all the three. Vitis vinifera showed 7 mm of activity against Trichophyton rubrum, no activity aginst Trichophyton mentagrophytes and Trichophyton tonsurans. Curcuma longa showed a slightly higher activity with 15 mm against Trichophyton rubrum, 12 mm against Tichophyton mentagrophytes and was resitant against Trichophyton tonsurans. Trachyspermum ammi and Trigonella foenum graecum was resistive to all the three strains. Nerium oleander recorded with an activity of 8.5 mm against Trichophyton rubrum and 12.5 mm against Trichophyton tonsurans. While, it was resistive to Trichophyton mentagrophytes [34].

Similarly, in an another study on important medicinal plant Allium sativum using the methanolic fractions reported with a lesser activity of 12 mm against Trichophyton rubrum and was resistive against Trichophyton mentagrophytes and Candida albicans. Similarly the methanol fractions of Cymbopogon martinii showed a lesser activity of 10 mm against Trichophyton rubrum and totally resistive against Trichophyton mentagrophytes and Candida albicans. Highly valuable medicinal plant Catharanthus roseus was even recorded with negative results against Trichophyton rubrum, Trichophyton mentagrophytes and Candida albicans [35]. All the plants discussed above were shown for lesser activity comparing to the activity noted with the petals of Rosa bourboniana harvested from the vermicompost treated plot. However samples from the inorganic and control plot were recorded with mild activity which was comparatively lesser in activity than the vermicompost treated plot.

The minimum inhibitory drug concentration for the studied dermatophytes showed a vast difference among different samples obtained from different plots and between the studied strains. The MIC values for the samples from the T8 plot were in the range of 0.25 - 1.00 µg/ml, for the T7 plot it was 0.25 - 1.25 µg/ml and that of T1 was 0.50 - 1.75 µg/ml. Lower drug concentration for inhibiting the growth of dermatophytes confirms the rich content of antifungal compounds present in the samples of the organic plot. The positive control Clotrimazole inhibited dermatophytes in a MIC range of 0.25 - 0.125 µg/ml which is slightly lesser than that observed with the T2 plot. In spite of this limitation, chemical investigation of antifungal compounds present in petals of Rosa bourboniana is considered important because of the possibility of synthesizing these compounds or their analogues which may be used in the control of fungal diseases.

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